หอสมุดกลาง สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER IV

DISCUSSION

During exposure to one-third the MIC of oxacillin, cephalosporin, or penicillin, the number of cfu of *S. aureus* was significantly smaller for the treated cultures than the corresponding control. However, these cultures did show a continuous moderate increase in cfu during drug exposure. The cfu of *S. aureus* increased after exposure to one-fourth the MIC of lincomycin but was lower than the control[19]. Moreover, in 1975 Lorian had shown that the number of cfu of *S. aureus* increased only slightly during exposure to one-third the MIC of penicillin[18].

The present study supported the earlier reports. *S. aureus* and *P. aeruginosa* grew significantly from 4 to 16 hr in the presence of subinhibitory concentration ($\frac{1}{2}$, $\frac{1}{4}$ MIC) of ampicillin or gentamicin but the cfu per ml were lower than the controls. Moreover, the results from study also demonstrated that, from 16 to 24 hr *S. aureus* still grew significantly in the presence of these subinhibitory concentrations of antibiotics, and *P. aeruginosa* also apparently grew from 16 to 24 hr of incubation in the presence of one-half the MIC of ampicillin or gentamicin and one-fourth the MIC of ampicillin, but in the presence of one-fourth the MIC of gentamicin, its growth curve declined. These seemed to express that, *P. aeruginosa* incubated in the presence of one-half the MIC of ampicillin or gentamicin and one-fourth the MIC

of ampicillin, and *S. aureus* incubated in the presence of these subinhibitory concentrations of ampicillin or gentamicin, they adapted themselves to grow in these condition and the growing periods were longer than the control cultures. It might be conceivable that one-fourth the MIC of gentamicin did not markedly affect the growth of *P. aeruginosa*.

S. aureus exposed to subinhibitory concentrations of penicillin, turned into cells that were larger than the control cells and cells that showed abnormal shapes with oval and other irregular forms as well as thick and numerous cross walls[5,8,18,19,20]. The observations in the present study did not fully support these reports. In the presence of subinhibitory concentrations of ampicillin, not all of the bacterial cells were larger and more irregular in morphology than the control cells. Only some cells had those abnormal shapes and the percentage of abnormal cells was significantly reduced after prolonged incubation, these might due to the rugular dividing and separating of unaffected cells whereas the treated cells dividing but could not separate normally. Moreover, most of the treated cells had single wide cross walls. The thinness of the peripheral cell wall of ampicillin-treated cells seemed to cause by the stretching due to the increase of diameter of the cells without concomitant cell wall growth.

It had been shown that retention of the crystal violet iodine complex by bacterial cells was proportional to the amount of mucopeptide in their cell walls[59]. The presence of unusually

thick cross wall could thus be accounted for the greater retention of crystal violet observed on the Gram stain of the large staphylococci after exposure to subinhibitory concentrations of ampicillin.

The observation in the present study also supported earlier reports that in the presence of subinhibitory concentrations of penicillin, Gram-negative bacilli formed unsegmented filaments[5,8,16,17,24,25]. By analogy to previous suggestion, it was conceivable that the morphological changes produced in several microorganisms by subinhibitory concentrations of penicillin might be the result of inhibition of whatever enzymes contributed to lysis of septa during cell division. Separation of the new cells could not then occur, and elongated bacilli or irregular-shaped cocci would be resulted. Moreover, in 1974 Burdett et al[60] found that the elongation appeared to result from inhibition of autolytic enzymes that initiated separation. The distribution of the electron dense materials along the filaments explained the granular appearance of subinhibitory concentrations of ampicillin-treated *P. aeruginosa* after Gram staining.

Gentamicin inhibited protein synthesis and had not been shown to interfere with cross wall or cell wall synthesis[36], so the morphology of gentamicin-treated cells did not markedly differ from the control cells when observed on the Gram staining smears. *S. aureus* exposed to subinhibitory concentrations of gentamicin, resulted in some rather round cells and in some larger cells with two convex cross walls which had no central dense layer when observed by electron microscopy, these could explain the

appearance of diplococci on the Gram staining smears. *P. aeruginosa* exposed to subinhibitory concentrations of gentamicin, resulted in vacuolation and in changing of density and distribution of the ribosomes, the latter could explain the bipolar appearance on the Gram staining smears.

In 1975 Lorian[18] found that, after growing for 2 hr on drug-free agar of the culture that had been exposed to penicillin before, *S. aureus* still exhibited abnormal forms, but some cells in normal appearance could also be observed. The present study, after growing for 18 hr in drug-free TSB of such ampicillin pre-exposed cultures, *S. aureus* still exhibited abnormal morphology and many cells were in normal appearance. Moreover, these cultures were markedly more resistant to ampicillin and were also more resistant to gentamicin than normal in some condition. Some cultures of *S. aureus* that grew for 18 hr in drug-free TSB after their exposure to gentamicin were more resistant to ampicillin and gentamicin than normal, though most of them were in normal appearance when observing on the Gram staining smears. These observations supported the appearance in strains of drug resistance isolated from pathologic processes has followed the introduction of the various antibiotics in general use[30].

Many cells of *P. aeruginosa* that grew for 18 hr in drug-free TSB after exposure to ampicillin still exhibited abnormal forms and some of these cultures were more resistant to ampicillin and gentamicin than the control. Cultures of *P. aeruginosa* that grew for 18 hr in drug-free TSB after exposure to gentamicin were

also more resistant to gentamicin but not to ampicillin, though their morphology was resemble the control observing on the Gram staining smears.

From these overall studies, it was conceivable that the pre-exposure to effective antibiotic of the microorganisms, in some conditions, might result in the resistance strain of such organisms to that antibiotic and/or the others, even in low concentration of subinhibitory levels of antibiotics and in the pathologic processes of the microorganisms.